inhibition of store-operated calcium entry (SOCE). Here we use patch-clamp analysis, TIRF and confocal microscopy, Ca^{2+} imaging and biotinylation experiments to systematically investigate whether Orai2 or Orai3 are capable of forming true store-operated or store-independent channels by themselves or whether and when they require Orai1. Using Jurkat T cells stably expressing Orai homologs, we track localization to the immunological synapse, determine redox sensitivity of SOCE and using concatenated constructs, unravel the stoichiometric requirements for redox inhibition of heteromeric channels. Our results demonstrate a dominant requirement for the presence of Orai1 to escort and confer store-operated activity onto Orai3 subunits and vice versa a dominant requirement for Orai3 to yield redox insensitive heteromeric SOCE complexes with Orai1.

1612-Pos Board B342

Icrac in Human Primary Prostate Epithelial Cells

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Expression levels of membrane androgen receptors (mAR) are elevated in prostate cancer and correlate with a negative prognosis. Rapid androgen response in prostate cancer can counteract several cancer hallmark functions such as unlimited proliferation, enhanced migration, adhesion and invasion and the inability to induce apoptosis. Thus mAR have been proposed as targets for therapeutic strategies. However, the molecular identity and downstream signaling pathways of mAR are still elusive. In primary cultures of human prostate epithelial cells from non-tumorous tissue (hPEC), we identified a rapid 5alpha-dihydrotestosterone (DHT) induced activation of store-operated Ca^{2+} entry.

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Activation of STIM1 by L-Glutamate Rapidly Inhibits L-Type Calcium Channel Current in Cultured Hippocampal Neurons

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At glutamatergic synapses, synaptic activity elicits postsynaptic Ca^{2+} signals that are generated in part by voltage-gated, L-type calcium channels. L-channel Ca^{2+} signals are known to control transcription of genes involved in synaptic plasticity, such as during late-phase long-term potentiation. Here we report that, in short-term cultured rat hippocampal neurons, 15-sec application of L-glutamate inhibited pharmacologically-isolated L-type Ca^{2+} current: inhibition ensued with a $t_{1/2}$ of 49 $\pm$ 2 sec, and current was reduced by ~35%. Measurements made with a genetically-encoded Ca^{2+} indicator (D1ER) targeted to intracellular stores showed that application of L-glutamate triggered release of Ca^{2+} from stores. FRET-imaging demonstrated that L-glutamate application also induced interaction of the Ca^{2+} store-depletion sensor STIM1 (CFP-tagged) with the L-channel scaffolding protein, AKAP79/150 (YFP-tagged), but not another AKAP scaffold protein, AKAP15/18. The time course of L-channel inhibition paralleled that for the increase in STIM1-AKAP79/150 FRET, suggesting a functional relationship between glutamate-triggered STIM1 translocation and inhibition of L-channel current. Supporting this idea, RNAi knockdown of STIM1 abolished glutamate-induced inhibition of L-channel activity. The ability of glutamate to inhibit L-channels in STIM1-knockdown neurons was rescued by co-transfection of rat neurons with the human STIM1 isotype, which was insensitive to the rat RNAi. Our results suggest a model wherein (i) glutamate receptor activation triggers Ca^{2+} release from endoplasmic reticulum (ER) stores within seconds of glutamate application, (ii) lowered ER Ca^{2+} activates STIM1 translocation to ER/plasma membrane junctions, and (iii) STIM1 interaction with the L-channel-AKAP79/150 complex down-regulates L-type Ca^{2+} current. This STIM1-mediated link between glutamate receptors and L-type Ca^{2+} channels may tune cytoplasmic Ca^{2+} signals involved in synaptic plasticity of hippocampal neurons.

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Characterization of Store-Operated Calcium Channels in Pancreatic Duct Epithelia

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Store-operated Ca^{2+} channels (SOCs) are activated by depletion of intracellular Ca^{2+} stores following agonist-mediated Ca^{2+} release. In pancreatic duct epithelial cells (PDEC), the activation of GPCR coupled to phospholipase C stimulated SOC-mediated Ca^{2+} influx. Direct measurement of $[Ca^{2+}]$ in the ER showed that SOCs slowed ER depletion. SOC-mediated currents were inwardly rectifying and greatly increased in the absence of divalent cations, as typical for SOCs in other cell types. Pharmacology of epithelial SOCs was consistent with that of some types of SOCs. In polarized PDEC, SOCs were localized specifically to the basolateral membrane. Both STIM and Orai proteins were expressed in PDEC and were colocalized after store depletion. Furthermore, knockdown of Orai3 expression, the most abundant Orai subtype, reduced SOC-mediated Ca^{2+} influx significantly. In conclusion, basolateral Ca^{2+} entry through SOCs fills internal Ca^{2+} stores depleted by external stimuli and facilitates Ca^{2+}-dependent cellular processes such as salt and mucin secretion from the exocrine pancreatic ducts.

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Cholesterol Regulates Orai1 Function

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STIM1 and Orai proteins represent the essential molecular components of Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Following ER-store depletion, CRAC current activation occurs due to the physical interaction between the STIM1 C-terminus and Orai1 N- and C-termini. Here we focused on an additional role of the extended transmembrane Orai1 N-terminal (ETON) region in cholesterol binding as it contains a cholesterol binding motif. Both chemically induced cholesterol depletion as well as point mutations disrupting the Orai1 cholesterol binding site enhanced store-operated Orai1 currents about 2-fold. Currents were not increased due to enhanced plasma membrane expression as revealed by biotinylation experiments. In addition, Orai1 point mutants that disrupted the cholesterol binding motif were not anymore sensitive to chemically induced cholesterol depletion. In accordance, employing intrinsic fluorescence measurements we detected direct binding of cholesterol to an N-terminal fragment containing the cholesterol binding motif with an equilibrium dissociation constant (K_D) of about 2µM, while mutations disrupting it increased the K_D 3-5 fold compared to wild-type. In aggregate, we propose a modulatory role of cholesterol on CRAC channel function. (supported by the Austrian Science Fund: FWF project P25210 to I.D., FWF project M01506000 to I.J. and FWF project P25172 to C.R.)

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Orai and TRPC Channel Contribution to Calcium Signaling in Human Mast Cells

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Mast cells are important targets for the treatment of allergic diseases and anaphylaxis. Inappropriate and chronic activation of mast cells via the IgE receptor leads to the release of a range of pre-stored and newly synthesized inflammatory mediators and the symptoms of disease. Calcium influx is a critical regulator of mast cell signaling, with influx through ion channels absolutely required not only for the exocytosis of preformed mediators but also to direct the synthesis of eicosanoids, cytokines and chemokines. Studies in rodent and human mast cells have identified STIM-regulated Orai channels to be key players in initiating calcium influx and degranulation in antigen-stimulated mast cells; in rodent mast cells, a role for TRPC channels is also emerging. Here we report evidence for a role of TRPC channels in human mast cell signaling. Using gene microarray analysis, we find evidence for expression of multiple TRP family members in primary human lung mast cells and LAD2 cells; the expression of TRPC1 and TRPC6 was further confirmed by immunocytochemistry. Single cell fura-2 imaging experiments, showed that barium could partially substitute for calcium to support influx following antigen- and thapsigargin stimulation, consistent with a contribution of non-selective TRPC-like channels to store operated calcium entry in human mast cells. To investigate the potential involvement of TRPC1 in STIM-regulated store operated calcium signaling in human mast cells, LAD2 cells were transfected with the STIM1 (684KK685) TRPC1 gating mutant. Expression of the mutant, however failed to alter calcium signaling in either thapsigargin or antigen-stimulated cells. Further pharmacological and molecular experiments are currently being performed to further evaluate the putative role of other TRPC channels in human mast cell signaling.